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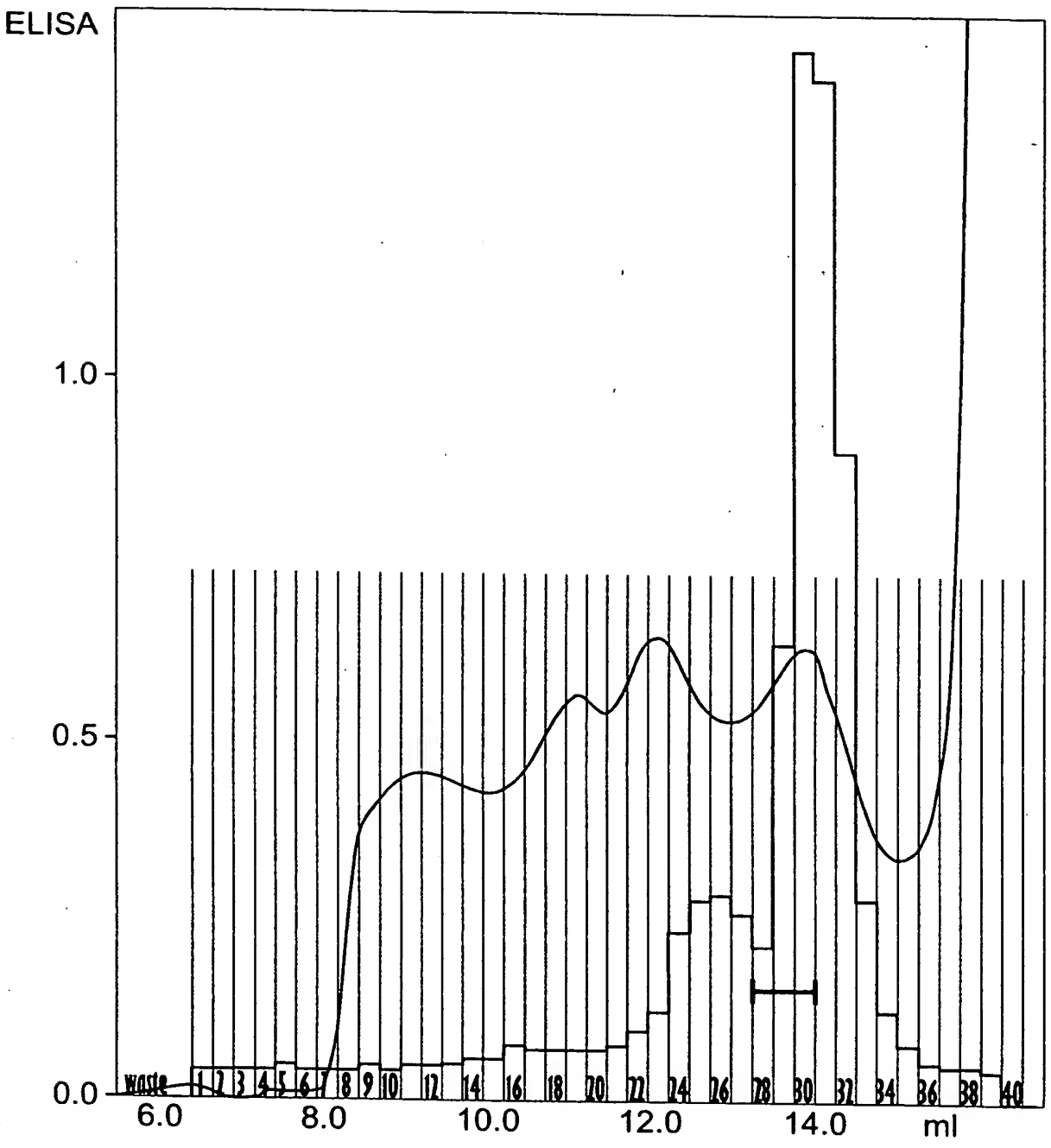
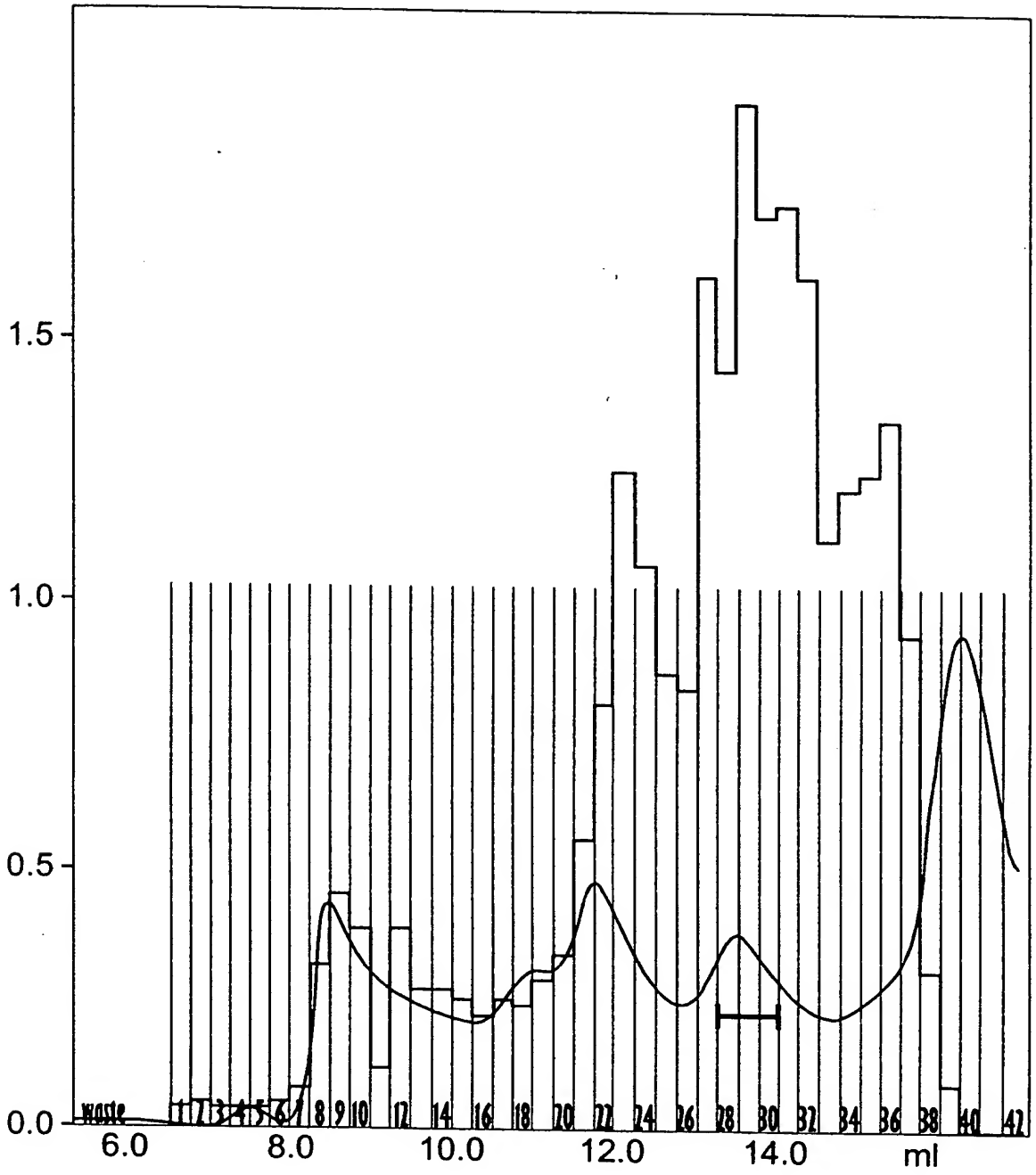

ELISA
1.0
0.5
0.0
waste
1
2
3
4
5
6
7
8
9
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
6.0
8.0
10.0
12.0
14.0
ml

Figure 1A


1.5
1.0
0.5
0.0
waste
1
2
3
4
5
6
7
8
9
10
12
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16
18
20
22
24
26
28
30
32
34
36
38
40
42
6.0
8.0
10.0
12.0
14.0
ml

Figure 1B

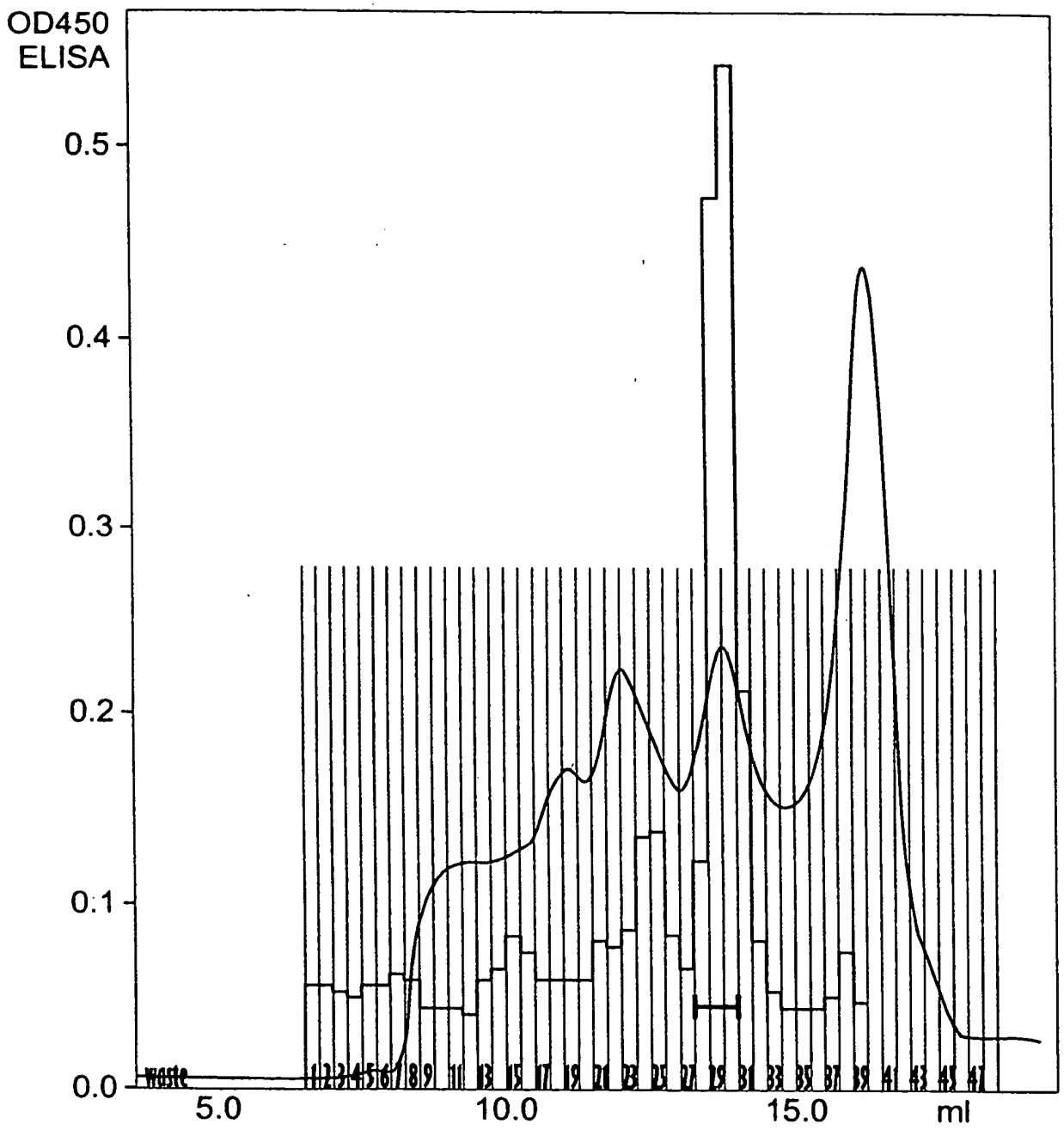


Figure 1C

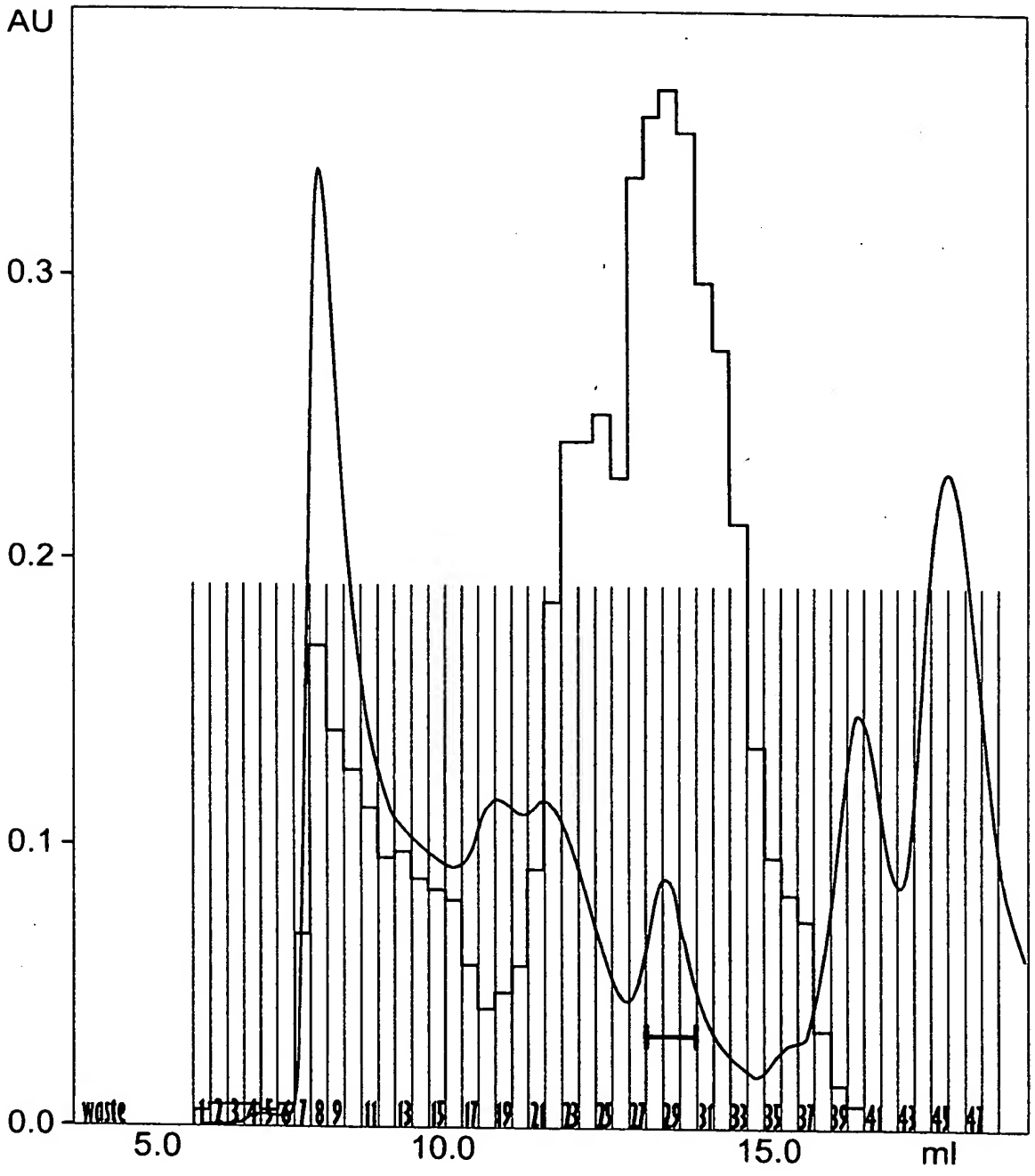


Figure 1D

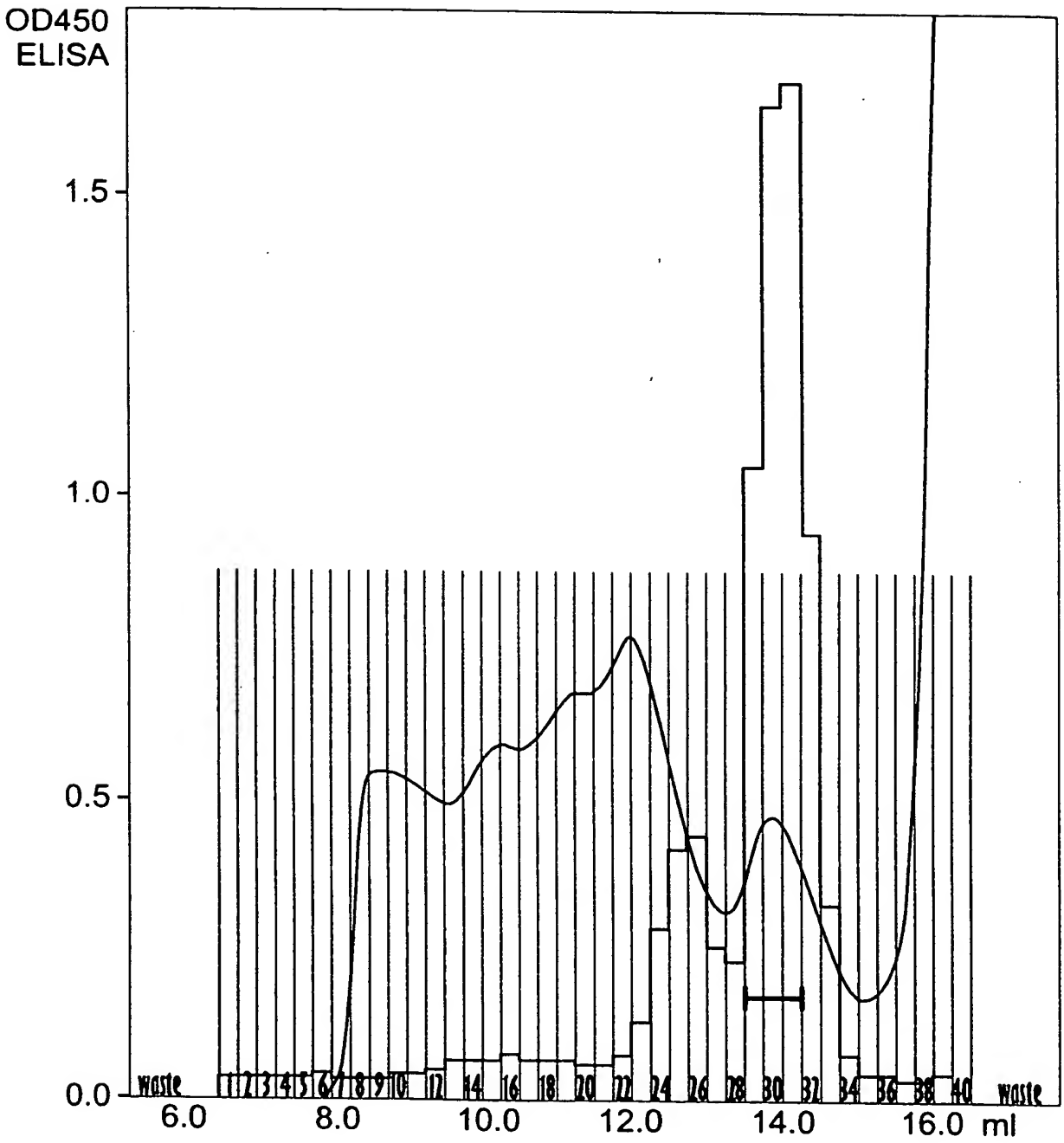

OD450
ELISA
1.5
1.0
0.5
0.0
waste
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
waste
6.0
8.0
10.0
12.0
14.0
16.0 ml

Figure 2A

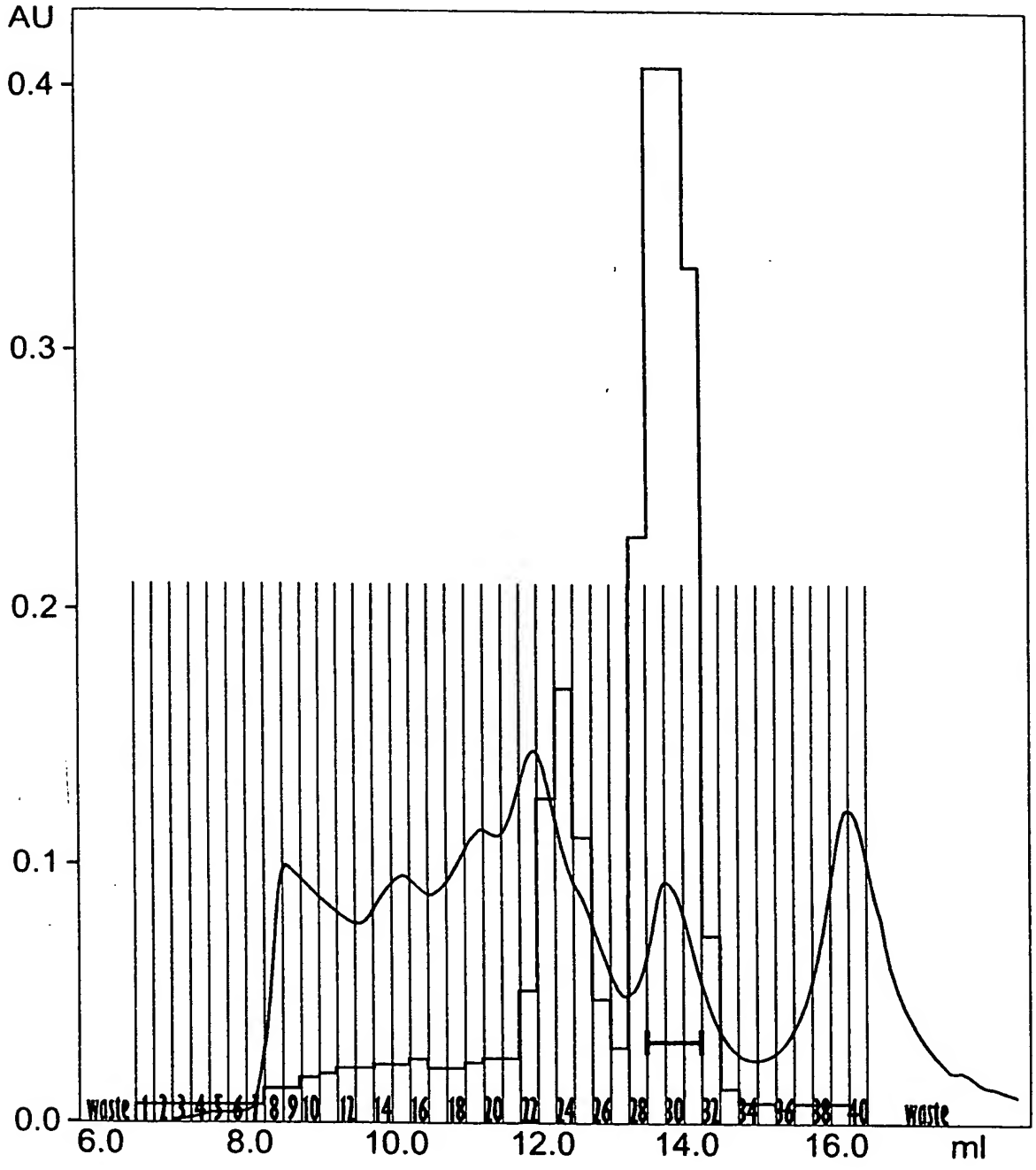


Figure 2B

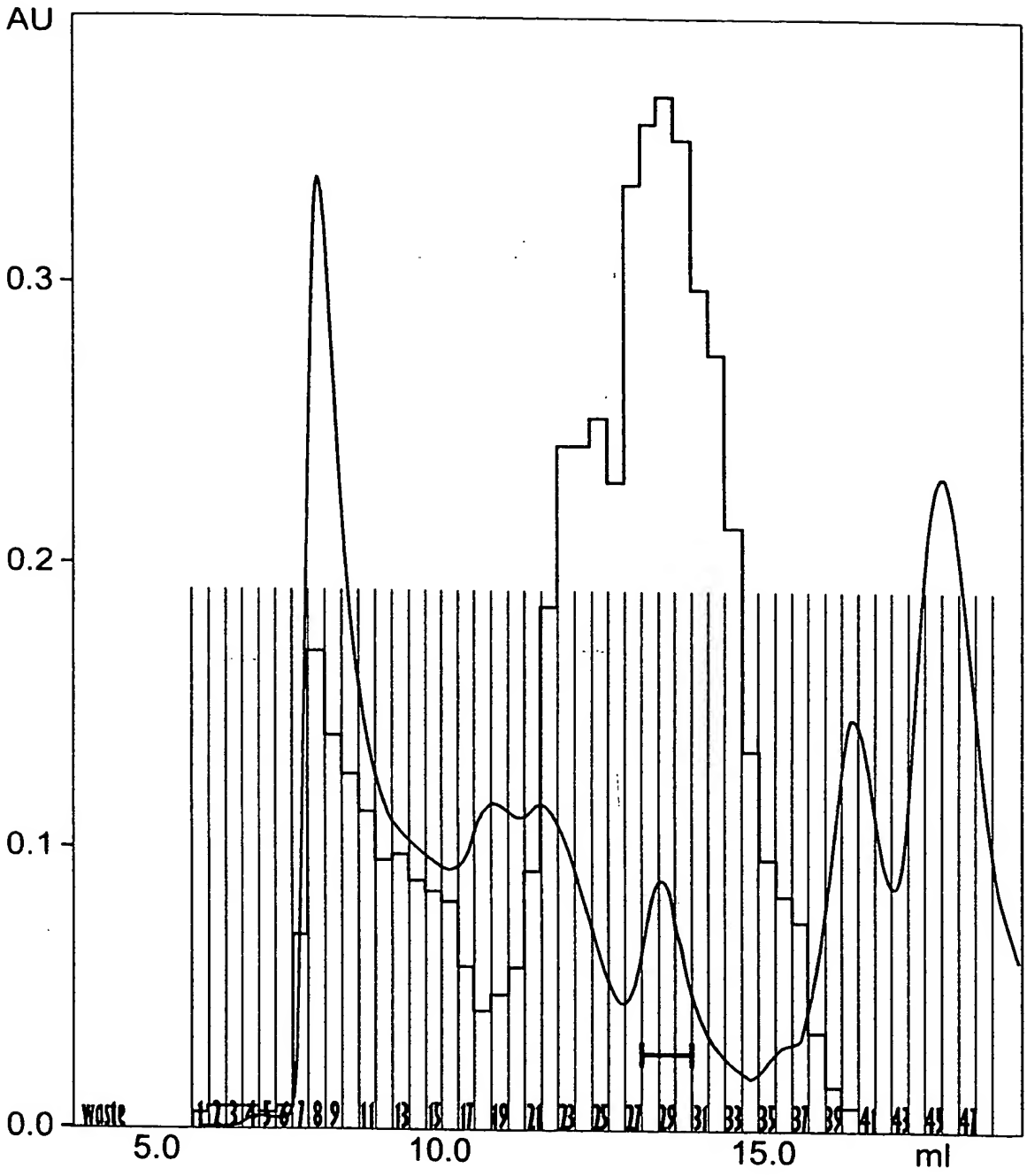


Figure 2C

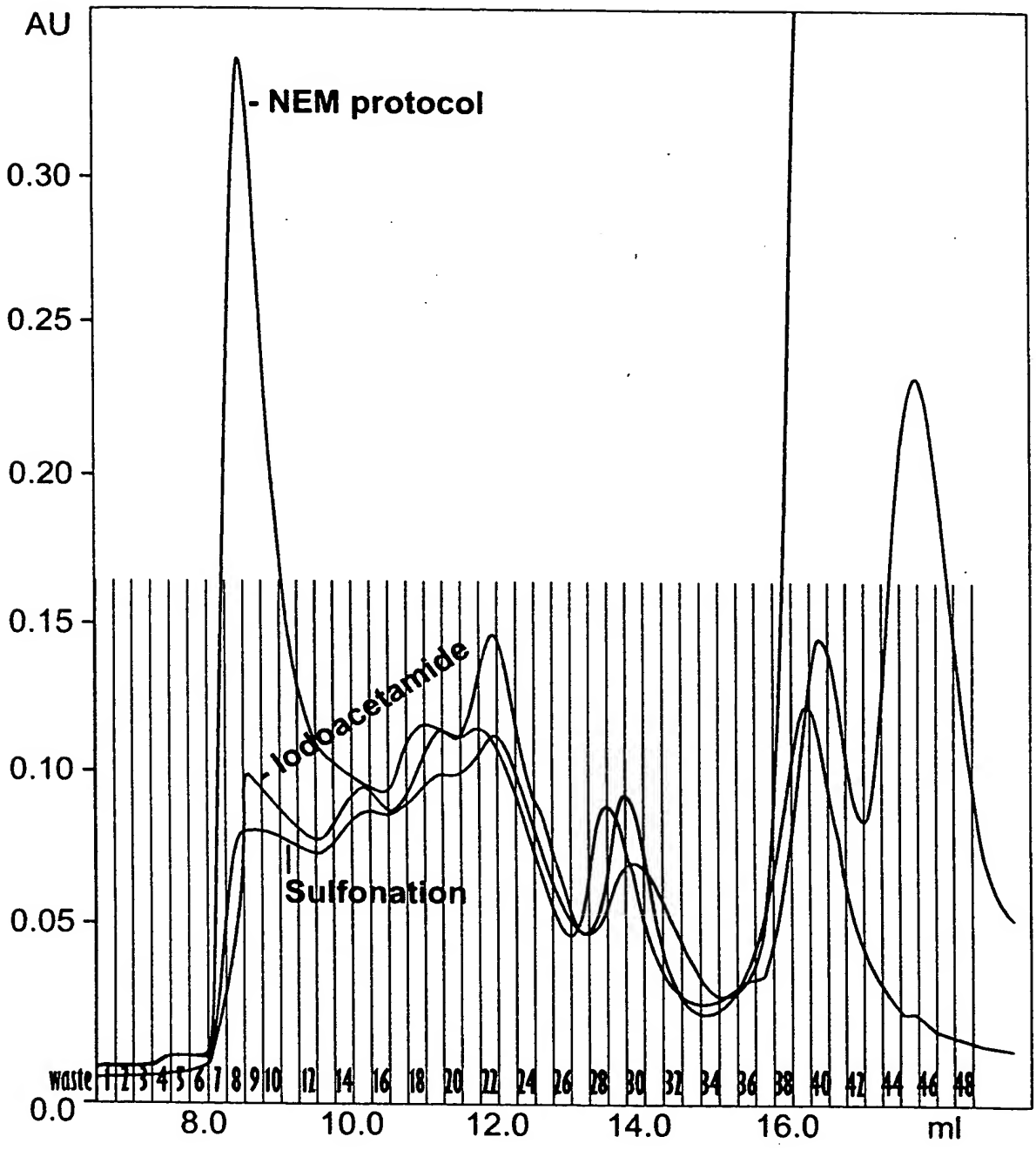

AU
- NEM protocol
0.30
0.25
0.20
0.15
0.10
0.05
0.0
- Iodoacetamide
Sulfonation
waste 1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
8.0
10.0
12.0
14.0
16.0
ml

Figure 2D

SILVER STAINING: SCREENING FRACTIONS SEC 3% EMPIGEN

Lane 1 and 10: Markers
Lane 2 till 5: Lysis Ascorbate, DTT reduction without blocking
Lane 6 till 9: Lysis Ascorbate, DTT reduction with sulfonation

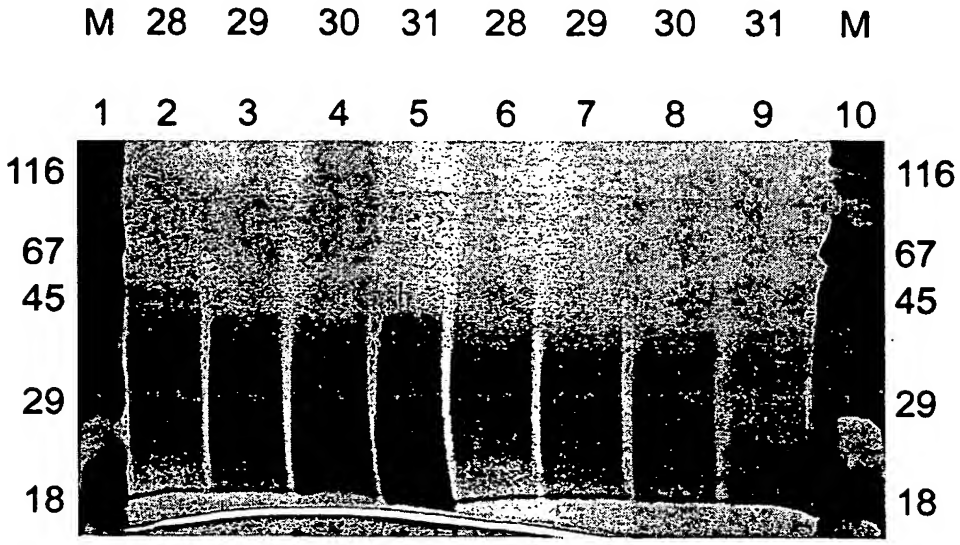


Figure 3a-1

MAB ANTI-E1 BLOT
SCREENING FRACTIONS SEC 3% EMPIGEN

Lane 1: Markers
Lane 2 till 5: Fractions SEC Lysis ascorbate, DTT reduction, ascorba

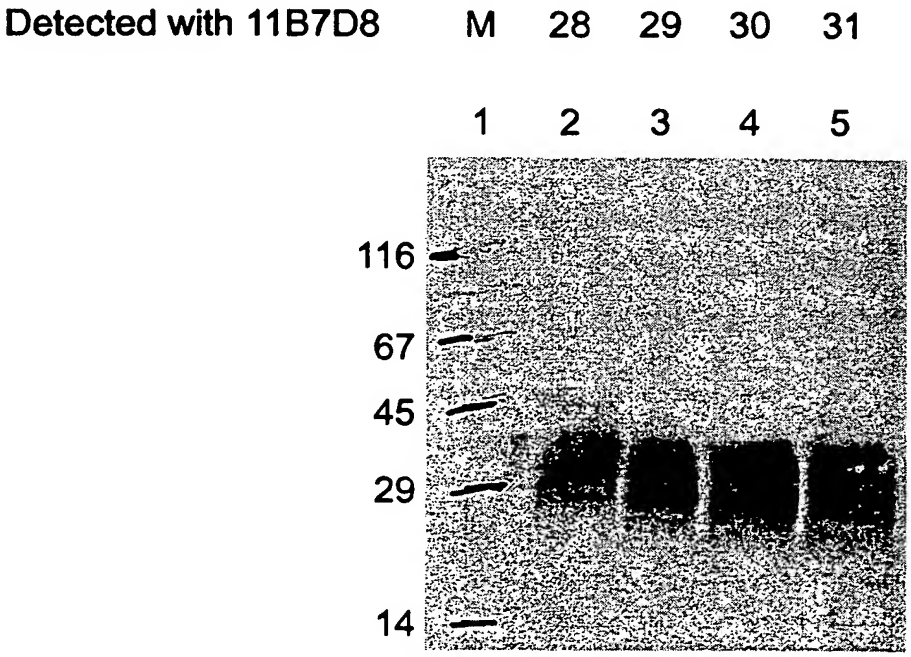
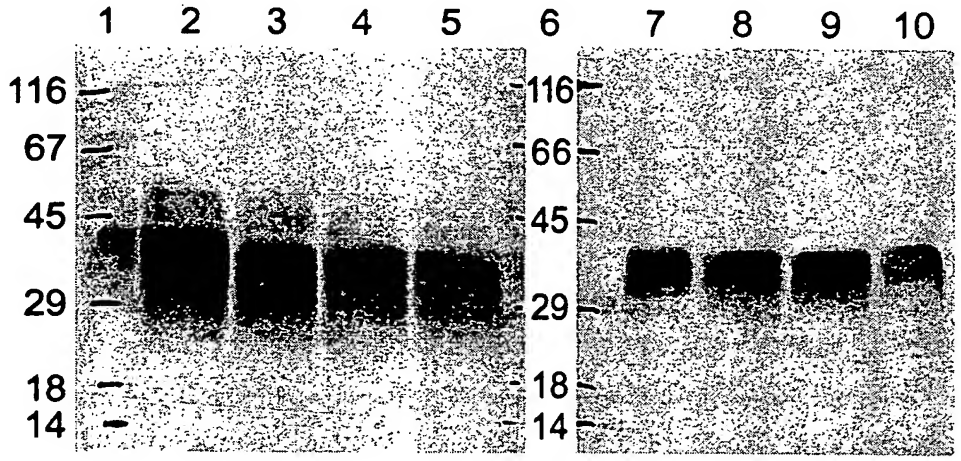


Figure 3a-2

MAB ANTI E1 BLOT
POOL SEC 3% EMPIGEN

Lane 1=6 Molecular weight markers
Lane 2=7 Pool SEC NEM protocol
Lane 3=8 Pool SEC IAA protocol
Lane 4=9 Pool SEC Lysis sulfonation, DTT reduction + sulfonation
Lane 5=10 Pool SEC Lysis ascorbate, DTT reduction + sulfonation

Detected with: 11B7D8 5E1A10



MAB 11B7D8: B epitope (amino terminale)
MAB 5E1A10: A epitope (carboxy terminale)

Legend lanes:

NEM protocol: lysis in presence of NEM, DTT reduction and alkylation with NEM
IAA protocol: lysis in presence of IAA, DTT reduction and alkylation with IAA
SO_3+SO_3: sulfonation after lysis, DTT reduction and sulfonation
ASC + SO_3: lysis in presence of Lascorbate, DTT reduction and sulfonation

Figure 3b

Purification of mTNF $(His)_6$ NS3 B9

Cell lysis in 6M Gu.HCl

Sulfonation

IMAC on Ni-IDA

Pure NS3-SO_3^-

Treatment with 200 mM DTT

Desalt at pH 4

Dilute till 500 µg/mL

Store at - 70° (+/- AO)

ELISA: Dilute --> 0.5 µg/mL

(+/- AO)

Figure 4

ANTIOXIDANT GROUP I						
ANTIOXIDANT		SAMPLE DIL.	SERUM N°			
- 70°	Dilution	DTT (10 mM)	17790	17832	17826	17838
-	+	-	38	79	1067	1138
-	+	+	1675	2134	2187	2190
+	+	-	43	59	1051	1059
+	+	+	1938	2175	1986	2155

ANTIOXIDANT GROUP II						
ANTIOXIDANT		SAMPLE DIL.	SERUM N°			
- 70°	Dilution	DTT (10 mM)	17790	17832	17826	17838
-	+	-	150	277	1739	2152
-	+	+	2064	2444	2474	2456
+	+	-	116	229	1564	1854
+	+	+	2095	2420	2509	2321

CONTROL	SERUM N°			
	17790	17832	17826	17838
NS3 B9 + 200 Mm DTT	938	1793	1802	1996
NS3 B9 + 10 Mm DTT	74	104	1874	2075

Figure 5a

Thiol Compounds and NS3B9 reactivity

Antioxidant		Reactivity of Serum N°(*)			
Cone (-70°,	(mM) Dilution)	17780	17790	17832	17801
-70° Dilution	---------- AO I + 1 mM GSH	353	1160	2026	1988
-70° Dilution	---------- AO I + 5 mM GSH	287	1087	1816	1850
-70° Dilution	AO I + 1 mM GSH AO I + 1 mM GSH	525	1384	2137	2194
-70° Dilution	---------- AO I + 1 mM Cys	287	935	1679	1712
-70° Dilution	---------- AO I + 5 mM Cys	299	1160	1757	1764
-70° Dilution	AO II AO II	603	1763	2396	2183
-70° Dilution	---------- 4 mM DTC	453	1389	2060	1963
-70° Dilution	---------- 4 mM Mono-SH	130	649	1396	1541

(*): Sample diluent in 3 mM DTT

AO I: 1 mM EDTA, 1 mM ascorbate
AO II: 2mM Mono-SH + 2 mM DTC
2 mM Mono-SH = 1mM TPCB + 1 mM TEG
2 mM DTC = 1mM DETC+ 1mM PDTC

GSH, Cys are reduced glutathion and cystein respectively

Figure 5b

NS3B9 B960925II

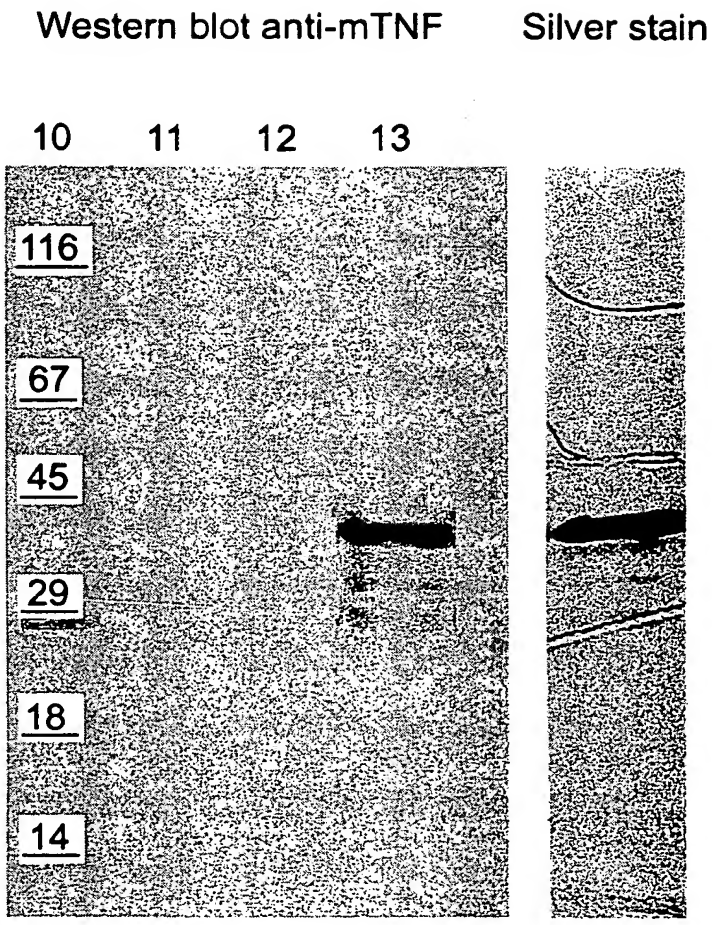
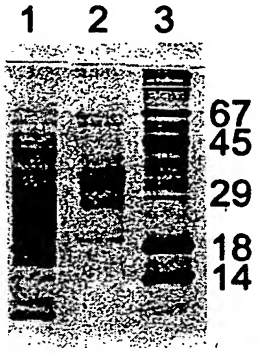


Figure 6a

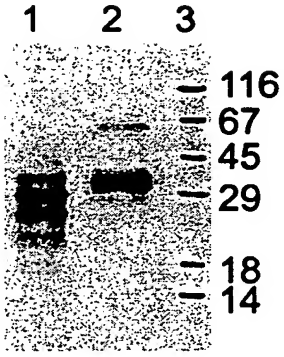
HIS-tagged E1 purified from *Saccharomyces cerevisiae*

Lane 1: E1 from *S. cerevisiae*
Lane 2: E1 from mammalian cells (vaccinia expression system)
Lane 3: marker proteins (M_r indicated in kDa)

A) Silver staining



B) anti-E1-blotting



C) GNA-blotting

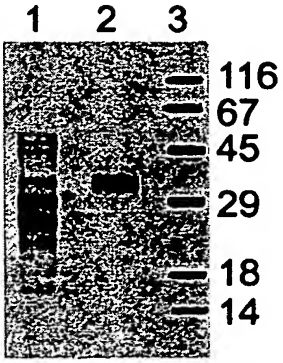


Figure 6b